

Effect of Electric Cigarette Smoke Exposure on Spermatozoa quality of mice (*Mus musculus* L)

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Received: 29 Oct 2021,

Received in revised form: 10 Dec 2021,

Accepted: 19 Dec 2021,

Available online: 27 Dec 2021

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Keywords— *E-cigarette*, *Spermatozoa*
quality, *mice*.

Abstract— *E-cigarette is one of the cigarettes that many used. E-cigarette smoke can cause disturbance in reproduction system. Chemical contents in e-cigarette smoke can increase free radicals that can cause oxidative stress. This purpose of this study to know the effect of e-cigarette smoke exposure to spermatozoa quality of mice (Mus musculus L). The tested animal were divides into 4 groups, every group consists of 6 mice that given treatment that is e-cigarette smoke exposure with a volume of L ml, 2 mL, and 4 mL for 4 weeks. Nicotine content in e-cigarette liquid was 6mg/mL. The parameter observed were percentage of motility and abnormal spermatozoa morphology. The conclusion of this study is e-cigarette smoke exposure decreased spermatozoa quality that is increased abnormal spermatozoa morphology in the treatment of e-cigarette liquid volume of 2 mL and 4 mL, meanwhile in spermatozoa motility is tend to decrease.*

I. INTRODUCTION

Cigarettes are one of the tobacco products that can cause health problems and even death for humans. E-cigarettes cause disorders of the respiratory system, cardiovascular system, hormonal imbalances, cancer and disorders of the reproductive system (Szumilas *et al.*, 2020). E-cigarettes are tools used by smokers to vaporize the liquid of an electric cigarette and the resulting vapor resembles tobacco smoke. This e-cigarette consists of several parts, namely the *battery* (the part that contains the battery), the *atomizer* (the part that heats and evaporates the nicotine and propylene glycol solution) and the *cartridge* (contains the e-cigarette liquid) (Electronic Cigarette Association, 2009). The chemical content of e-cigarette smoke causes an increase in free radicals which will cause oxidative stress (Sari, 2014). El Golli *et al.* (2016), Wistar rats that were treated with an e-cigarette liquid at a dose of 0.5 mg nicotine/kg BW/day intraperitoneally (IP) for 4 weeks could increase oxidative stress by producing ROS due to decreased testicular lactate dehydrogenase activity. In addition, there is also

interference with steroidogenesis and spermatogenesis.

E-cigarette liquid contains nicotine, water, propylene glycol, glycerol, and flavoring agents (Oroh *et al.*, 2018). Based on research conducted by the US Food and Drug Administration (FDA) in 2009, e-cigarette smoke contains Tobacco Specific Nitrosamine (TSNA) and Diethylene Glycol (DEG) which are toxic and carcinogenic (Alawiyah, 2017). Further research is needed regarding the effect of exposure to e-cigarette smoke on the quality of spermatozoa in male mice (*Mus musculus* L), including motility and abnormal spermatozoa morphology.

II. MATERIALS AND METHODS

2.1 Material of research

The materials used in the study were male mice (*Mus musculus* L) obtained from the the Center for Veterinary

Farma (Pusvetma), Surabaya, broiler pellet feed (BR1 Plus), the liquid used for electric cigarettes with strawberry

oatmeal flavor containing a dose of 6 mg of nicotine per mL, distilled water, chloroform, 0.9% NaCl, 2% formalin, Eosin as dye and entelan.

2.2. Procedure of research

Mice were divided into 4 groups, each using 6 mice as repetitions. Group 1 (without exposure to e-cigarettes smoke), group 2 (exposure to electric cigarette smoke with a liquid volume of 1 mL), group 3 (exposure to electric cigarette smoke with a liquid volume of 2 mL), and group 4 (exposure to e-cigarette smoke 4 ml). E-cigarette liquid contains 6 mg nicotine/mL. Exposure to e-cigarette smoke in group 2 was done once, in group 3 it was done twice, while group 4 was 4 times. After each exposure, the mice were rested for 15 minutes until the smoke disappeared and then proceeded to the next exposure. Exposure to cigarette smoke was carried out for 4 consecutive weeks by inhalation in a vaping box (El Golli *et al.*, 2016). Test animals were killed on day 28, one hour after the last treatment. Mice were anesthetized using chloroform before surgery to remove the epididymis. The left cauda epididymis was put into a watch glass containing 0.9% NaCl solution to observe sperm quality.

Spermatozoa quality observations were carried out to determine the motility and morphology of mice spermatozoa. Spermatozoa quality observation methods were as follows:

a. Spermatozoa Motility

Spermatozoa suspension (cement) in 0.9% NaCl was taken with a pipette and one drop of suspension was placed on a hemacytometer then covered with a cover slip and observed under a microscope with a magnification of 400 times. Spermatozoa calculation was carried out from one field of view on ± 300 spermatozoa and then classified to produce the percentage of each motility category. Category motility was as follows:

- 1) Category 0 = sperm do not move at all
- 2) Category 1 = spermatozoa moving very slowly
- 3) Category 2 = spermatozoa moving forward with moderate speed / moving zig-zag and swirling
- 4) Category 3 = spermatozoa move straight fast forward.

Percentage of motility of spermatozoa was determined by the formula:

$$\% \text{ motility} = \frac{\text{kategori } 2+3}{\text{kategori } 0+1+2+3} \times 100\%$$

(Soehadi and Winarso, 1987).

b. Abnormal spermatozoa morphology

The cement suspension was placed one drop on an object glass, then 2% formalin was added and dried. After that, one drop of 1% Eosin was given and then rinsed with distilled water and then covered with a cover glass and cooled down. Further observations under microscope with a magnification of 400 times (Arsyad and Hayati, 1994).

Observations were made on 3 fields of view, counted the number of normal spermatozoa and abnormalities of spermatozoa which include abnormalities of the head, middle and tail (Arsyad and Hayati, 1994). Observation results are expressed in percentage with the formula:

$$\% \text{ abnormal} = \frac{\text{Jumlah spermatozoa abnormal}}{\text{Jumlah spermatozoa normal+abnormal}} \times 100 \%$$

(Toelihere, 1993)

III. RESULTS AND DISCUSSION

The average percentage of sperm motility and morphology of abnormal spermatozoa in mice after exposure to e-cigarette smoke is presented in Table 1.

Table 1. Motility and abnormal spermatozoa morphology of Mice (*Mus musculus* L) after exposure to e-cigarettes

| E-cigarette liquid volume | Spermatozoa motility (%) ($\bar{x} \pm SD$) | Abnormal spermatozoa morphology (%) ($\bar{x} \pm SD$) |
|---------------------------|---|--|
| Control | 25.03 \pm 9.55 | 50.56 ^a \pm 9.50 |
| 1 mL | 24.73 \pm 13.10 | 55.01 ^{ab} \pm 5.81 |
| 2 mL | 22.25 \pm 10.79 | 59.48 ^b \pm 4.33 |
| 4 mL | 13.94 \pm 7.71 | 60.95 ^b \pm 4.75 |

Note: Numbers in the same column followed by the same letter show no significant difference)

The results of One Way Anova test, a significance value of $p = 0.253 > 0.05$ was obtained on the motility of spermatozoa. This indicates that exposure to e-cigarette smoke does not significantly affect the motility of spermatozoa. However, the higher the volume of e-cigarette liquid used, the lower the average sperm motility of mice.

The results of One Way Anova test on the average morphology of abnormal spermatozoa obtained a value of $F = 3.199$ with a significance of $p = 0.045 < 0.05$. This

shows that the volume of e-cigarette liquid has a significant effect on the morphology of abnormal spermatozoa. The results of the Duncan Multiple Range Test (DMRT) = 0.05 showed that the control was not significant with 1 mL e-cigarette liquid volume and significant with 2 mL and 4 mL e-cigarette liquid volumes. This shows that the administration of e-cigarette liquid with a volume of 2 mL and 4 mL has increased the percentage of abnormal spermatozoa morphology. This is presumably because the increased nicotine content causes an increase in free radicals.

In this study, the administration of e-cigarette smoke did not significantly affect the motility of spermatozoa although the motility of spermatozoa decreased with increasing dose. The decrease in spermatozoa motility is thought to be due to the influence of free radicals generated by e-cigarette smoke inhibiting oxidative phosphorylation in obtaining energy. Mitochondria are cell organelle that functions to produce energy (ATP) (Fitriani *et al.*, 2010). Mitochondria are targets for nicotine because they have nAChRs (Nicotinic Acetylcholine Receptors). Binding between nicotine and nAChRs might increase ROS, thereby reducing ATP production. The main source of reactive oxygen compounds (ROS) in cells also comes from the mitochondrial electron transport chain. Based on this, the decrease in spermatozoa motility occurred due to the decrease in ATP caused by the production of ROS by binding of nicotine to nAChRS. The motility of spermatozoa is influenced by the energy produced by the mitochondria in the neck of the spermatozoa (Bourgeron, 2000). According to Susmiarsih (2010), decrease in the quality (decrease in motility) of spermatozoa is caused by oxidative stress on spermatozoa so that it can inhibit the production of ATP produced by the mitochondria of spermatozoa through oxidative phosphorylation.

The motility of spermatozoa is highly dependent on the supply of energy in the form of ATP as a result of metabolism. Spermatozoa mitochondria located in the midpiece of spermatozoa function to produce energy (ATP), while the principal piece and end piece function in the movement of spermatozoa. ATP that has been synthesized in the mitochondria is transported to the axoneme at the tail of the spermatozoa, then converted by dynein (ATPase enzyme) in the axoneme which will decompose ATP into energy for the movement of spermatozoa. The inhibition of the release of ATP to the axoneme results in unfulfilled or reduced energy requirements to move the tail, which in turn results in reduced motility of spermatozoa or does not move at all (Astuti *et al.*, 2009).

ROS also causes an increase in abnormal spermatozoa morphology. In this study, the morphology of abnormal

spermatozoa increased with increasing dose. It is suspected that high ROS levels can cause damage to spermatozoa membranes. According to Sanocka *et al.* (2004), the plasma membrane of spermatozoa contains high levels of phospholipids, causing spermatozoa to be very susceptible to ROS. This indicates that spermatozoa membranes are the main target of ROS and lipids are potential targets (Lamirande *et al.*, 1997). Lipid oxidation (lipid peroxidase) in spermatozoa membranes produces malondialdehyde (MDA) compounds, which are toxic to cells, causing damage to spermatozoa membranes. According to Susilawati (2011), the function of the membrane is to protect the cell. Damage to the spermatozoa membrane causes a decrease in the integrity of the spermatozoa membrane and disrupts intracellular metabolic processes, which in turn causes a decrease in sperm quality and even death of spermatozoa. Damage to the spermatozoa membrane causes disruption of cell metabolism, thereby increasing the morphology of abnormal spermatozoa.

In this study, primary and secondary abnormalities were found. The primary abnormalities found in this study included forked head while the secondary abnormalities found in this study included a twisted tail, a bent tail, a bent neck, and a severed tail. Morphology normal and abnormal spermatozoa in mice found in this study can be seen in figure 1.

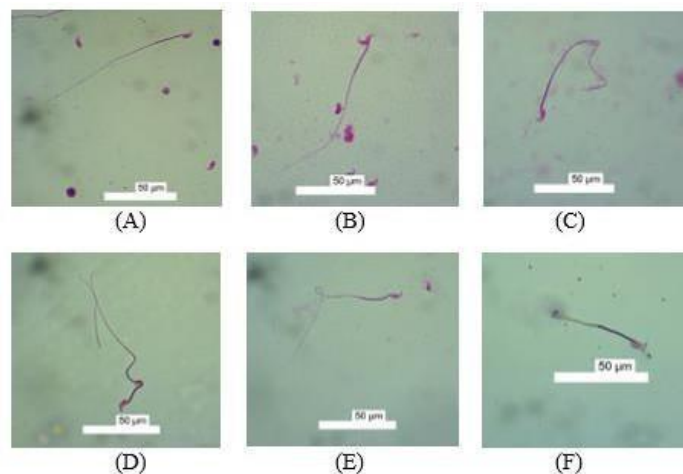


Fig.1. Morphology of normal spermatozoa (A), primary (B) and secondary (C - F) abnormalities with 400x microscope magnification

Primary abnormalities occur in the testes due to impaired function of Leydig cells in producing testosterone. Testosterone is needed in the process of spermatogenesis (Garner and Hafez, 2000). Secondary abnormalities occur in the epididymis. Disruption of Leydig cells also interferes with the sperm maturation process because if

testosterone is reduced, the epididymal epithelium will thin so that the compounds needed in the maturation process are reduced (Zhan *et al.*, 2012).

IV. CONCLUSION

Exposure to e-cigarette smoke decreased the sperm quality of mice by increasing the morphology of abnormal spermatozoa in the 2 mL and 4 mL e-cigarette fluid volume treatment, while the motility of spermatozoa tended to decrease.

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